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Translated by Sp/6 Charles T. Ostertag Jr.

Biochemical Classification of Piricularia Fungi - Producers of Physiologically Active Substances

V. I. Kefeli

Representatives of the genus Piricularia, which belongs to the class Fungi Imperfecti and the order Hyphomycetes, are extremely injurious to rice, bananas, wheat, cocoa and a number of wild grains and cause losses in the harvest of up to 70% and more. Recently Piricularia has become the object of an intensive study in various countries. Though quite a lot of experimental material has been accumulated about this fungus, up to the present time it still hasn't been systematized in our own literature. The present article is an attempt of a similar nature. Naturally in a short article it is not possible to check into all the problems of the morphology and physiology of this fungus. Therefore we will turn our attention to several of its biochemical properties.

The reasons for the intensive study of this parasite, which affects a number of important agricultural crops, are basically of an economic nature. Damage inflicted by Piricularia in southern countries can be compared with the disasters which are associated with the rusts of cereal grains in the north. Along with this, Piricularia, just like Gibberella fujikuroi, is a fungus which, while developing on rice or in an artificial nutrient medium forms a large amount of physiologically active substances. These substances represent the link which connects the metabolism of the host with that of the parasite which has taken root in it. A peculiarity of Piricularia is the very specific specialization of biochemical processes which permits the establishment of a clear classification of the species of the genus Piricularia and of the strains of P. oryzae. Before going into a description of the biochemical classification within the genus Piricularia, we will dwell on several features of this fungus and its relation with the rice plant.

I. General Information About the Pathogen

Piricularia was first described by Briosi and Cavara in 1892. Six years later in Japan, investigations began on the characteristics of this fungus which caused the disease "zapal" (premature drying of seeds) in rice.

The first phase of the investigations dates from the 1900s and was characterized by an intensive study of the relations of the plant and parasite in the plane of immunity.

In 1917 - 1928, a group of investigators headed by Nishikade isolated Piricularia from the leaves and stems of various plant-hosts. They studied, within the genus, the morphological-physiological peculiarities of these

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In 19[illegible]7 - 1928, a group of investigators headed by Nishikade isolated Piricularia from the leaves and stems of various plant-hosts. They studied, within the genus, the morphological-physiological peculiarities of these

isolates. In 1918 in India, the first strong epiphytotic was ascertained by McRae. The development of practical measures to combat the fungus began. New fungicides were tested and special stable varieties were uncovered. A whole series of works on the influence of fertilizers on the development of Piricularia was published by Thomas.

In 1928 a report appeared about the development of Piricularia in the Far East. The author of the article, I. N. Abramov, found that the leaves of rice are the most strongly affected organ. A dirty gray film develops on them. This is the beginning of the disease. Then the fungus shifts to the stems and other organs of the plants. It is possible that even part of the panicle is affected. The grain becomes quite sickly. Starch is almost lacking. The fungus causes a black mold of the lower nodes of the stem. The author points out that the Korean peninsula was probably the main focal point from where this disease spread out into the territory of the USSR. Before the Second World War, Japan and India were the main centers of investigation of Piricularia. Having obtained the physiological characteristics of the fungus, the Japanese microbiologists began to cultivate it under laboratory conditions. In the 30's and 40's the fungus was cultivated under laboratory conditions and the nature of its growth on artificial and natural nutrient media was studied (Leaver and others, 1947). A specific peculiarity of the Japanese investigations was the permanent control of the "fungus-host" interrelations. A whole series of microscopic analyses has been conducted in connection with this, for example, the investigations by Terui (1940) which demonstrated the interrelation between the fungous hyphae and the cells of the rice plant. The author pointed out that within the cells of the rice leaf, short spores are formed which are apparently the fertile source which allows the fungus to develop and hibernate. This trend of work was very important for perfecting measures for combatting Piricularia. Takachashi (1956) reported on the interesting results of microscopic investigations conducted on stable and sensitive varieties of rice. In the cells of leaves of stable varieties of rice, the fungous hyphae grew poorly, but a brown granularity appeared in cells of sensitive varieties and the hyphae from these cells sometimes spread to neighboring cells. Before the onset of the 40's, the countries of Europe and America were hardly acquainted with piriculariosis, however by 1950 a whole number of foci of rice affection were noted in the USA.

In connection with a strong epiphytotic, breeders in the states of Louisiana and Arkansas were forced to select the most stable varieties of rice (Crally and others, 1942; Atkins, 1956). In Hungary and Italy cases were also established (Corbetta, 1954; Vamos, 1959) of the spreading of piriculariosis, which compelled the Hungarian phytopathologists and physiologists to undertake the study of this disease. In this, more aerobic conditions were created for the root system of rice against a background of the various standards of nourishment; a similar measure should impede the development of the disease. The extensive spreading of Piricularia among the main rice producing countries led to a considerable increase of the front where combat with this fungus was being waged. Research centers specializing in piriculariosis were formed: 1) in India, the Botanical Laboratory of the University in Madrass, the Agricultural College in Poona,

and a number of institutions in Bombey; 2) in Japan, the Universities of Tokyo, Kyushu, Hokkaido, Nigata, and Yamagata; 3) in the USA, Fort Detrick, Maryland.

The main methods of research which are being carried out in these centers may be divided into three groups: 1) the physiology of the nourishment of the parasite; 2) biochemism of the interrelation of the fungus and the host; 3) active substances produced by the partners.

The main problem, connected with the physiology of nourishment of Piricularia, is especially detailed to work out. In the last 5 - 6 years this range of problems has emerged from the realm of bare empiricism; researchers began on the method of directed breeding of sources of nourishment necessary for the fungus. The greatest perspective was shown by the Indian series of works on the study of the reaction of the fungus to a concentration of hydrogen ions, nitrogen containing substances and vitamins (Apparao, 1955, 1956). By using highly purified media it was possible to find out that biotin and thiamine sharply intensify the growth of fungus. Microelements - iron, zinc and maybe copper are also necessary for Piricularia but are not synonymous in their effect. A detailed study of the vitamin nourishment of Piricularia compelled the Indian researchers to regard this fungus as one of a group of organisms heterotrophic in relation to vitamins. The vitamin requirement of P. oryzae is not confined to thiamine and biotin. The initial phases of growth (germination of spores, elongation of the germ tube) are stimulated by sterols, steride amines, a-tocopherol, and sapogenins (Weintraub, 1957, 1958).

The reaction of fungus to sources of nitrogen is connected to a whole series of attendant factors such as the pH, the reduction level of the compounds, etc. Leaver (1947) and Apparao (1956), while studying the growth of fungus in a medium with nitrate and ammonium salts, demonstrated their unequality. Apparao (1956) considers that the negative properties of ammonium salts depend on their physiological acidity. It must be noted that the optimum of activity of the basic ferments of fungus lies at a level of pH 5.9 to 7.3. The presence in the medium of some ammonium salts lowers the pH to 2.8 and this itself depresses the synthetic processes. Mineral sources of nitrogen generally are not the best for fungus and are replaced successfully by amino acids and peptone (Leaver and others, 1947, Ramakrishnan, 1948).

Carbon compounds on which P. oryzae grow are extremely various. Besides mono- and di-sugars, fungus can develop on cellulose, starch, pectin, and even on polyhydric alcohols, glycerin and mannitol (Ramakrishnan, 1948, Yamada, 1959). More recent investigations explained this phenomena by the capability of the fungus to form basic fermentation systems during cultivation, for example, on mannitol alone. Such an adaptive ferment in the last case turned out to be phosphatase of mannitol-1-phosphate, catalyzing the hydrolysis of mannitol, but not acting on its homologs. Another purified preparation, reductase of fructose-6-phosphate, controlled its reduction to mannitol-1-phosphate. The high synthetic and adaptive capability of the genus Piricularia and especially the species P. oryzae is explained

by the great activity of its fermentation apparatus. In table 1 there is presented (far from complete) a list of ferments, regulating the most important exchange processes of fungi (the table is taken from the work of Ramakrishnan, 1948).

The interrelations between the fungus and the plant is the second important problem which has been subjected to attentive study. It is necessary to dwell on this in as much as the circle of problems already treated has a direct relationship to the problem of the connection between parasite nourishment and host nourishment. It has been shown, for example, that fertilizing rice with nitrogen led to a spreading of the infection (Suryannarayanan, 1958a), and fertilizing with potassium decreased the damage to the rice (Corbetta, 1954). A leaf on which Piricularia is developing represents the same kind of active arena for fungus activity as the root zone for rhizosphere microflora. The leaves of stable varieties of rice subjected to infection form amino acids which are varied in quality and quantity. Stemming from this, Suryanarayanan (1958a) considers that the study of the biochemical mechanism of stability as a whole is a considerably more practical method for solving the problem of immunity then the biochemical study of genetically stable varieties.

The facts presented above are related to the reaction of the fungus on the macrocompounds of the plant-host. Bordering on this group of problems are the investigations for isolating physiological active substances. These works are the most promising since they disclose sharp reactions, originating during the penetration of the fungus into the leaf cells of the plant-host. The morphological side of this problem was elucidated earlier by Terui (1940) and Takachasi (1956).

We will examine a basic group of works concerning the biochemism of this phenomenon. There is data that a suspension from spores of P. oryzae, after it was aged on leave fragments, contained the toxic substance "phytoalexin" - a product of the interaction of the plant and the fungus. Phytoalexin was formed energetically (Uehara, 1958) on the leaves of stable varieties of rice. The interrelations of the fungus with the plant are not limited to the production of only one toxic source of the phytoalexin type. During guttation the rice plant yields a stimulating volatile substance "orizarol" which intensifies the germination of Piricularia spores. When isolated in a pure form, it maintained its activity even in subsequent experiments (Tamari, Kaji and others, 1958). Following orizarol other compounds were detected which possessed the capability of stimulating germination and the growth of spores (Tamari and others, 1960a, 1960b). These compounds turned out to be eugenol and its derivitives - eugenol -palmitate and isoeugenol. The physical constants, molecular weights, and structural formulas have been determined for all of them. With the help of paper chromatography these compounds are easily analyzed and determined chemically.

P. oryzae develops most intensively on a medium containing a nitrate source of nitrogen and saccharose. It has been found that namely in such

a medium the fungus synthesizes active substances similar to auxin. These substances were called growth hormone; they comprise 0.5% of the weight of dry vaccine isolated from P. oryzae. This hormone usually is extracted from the spores by chloroform and ether. It is capable of stimulating the growth of the roots and stems of rice. Paper chromatography has disclosed the similarity of this compound with indolyl-acetic acid (Watanabe, 1957).

Cultivations of P. oryzae on a medium with compounds which are difficult to assimilate (such as ammonium salt and starch) in conjunction with glucose and glutamic acid which are well absorbed by it lead to the increased formation in the cultured medium of a toxic complex, which includes a-picolinic acid and a specific nitrogen containing substance - piricularin (Tamari, Kaji, 1954). The components of piricularin, which were isolated from a cultured liquid, were also detected by chromatographic methods in the tissues of an infected plant (Tamari, Kaji, 1954).

We wil list the basic physiologically active substances detected in the study of the interaction of Piricularia and the rice plant during the period 1954 - 1960 (table 2).

Even a superficial acquaintance with the basic works on the biochemism of the processes which determine the interrelations between Piricularia and the rice plant disclose an unusually strong capability of the fungus to react to changes of components of the nutrient medium and to the biochemical composition of the host leaf tissue; thanks to this there takes place a rapid reorganization of the entire fermentation apparatus of the fungus, which is adaptable to changes of the medium. Making use of these properties of Piricularia and strengthening them with the help of selecting the nutrient media, they made attempts in India and Japan to classify species of the genus Piricularia and subsequently even strains of the species P. oryzae, having subdivided them into a number of clear groups with specific physio-biochemical properties. This classification assisted in the more complete study of the nature of the fungus in order to subsequently conduct a directed and systematic struggle against its spreading.

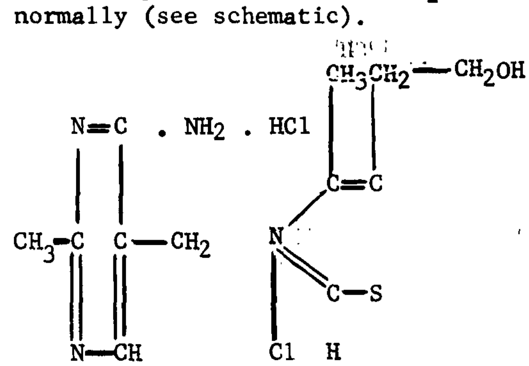
II. Classification of the Genus Piricularia According to Biochemical Features

A conception introduced by Beadle (1945) lies as the basis for the biochemical classification of the genus Piricularia and the species P. oryzae. This conception is built on the study of the requirements of a number of strains of Neurospora in new growth factors. "We propose - writes the author - that the initial strain may bring about the synthesis of the growth substance necessary for it and then a number of successive reactions, each of which is regulated by a specific ferment. This process can be schematically presented in this manner:

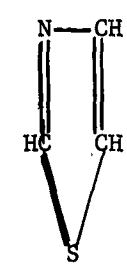
$$\text{SUBSTRATE} \xrightarrow[\text{Ferment 1}]{} P_1 \xrightarrow[\text{Ferment 2}]{} P_2 \xrightarrow{\text{Ferment 3}} P_3 \ldots\ldots P_p.$$

In accordance with this arrangement, a biochemically deficient mutant should grow similarly well with the addition to the medium of substance P_p or any of the intermediate products found in the chain of the reaction. P It turned out that mutants, destitute in one and the same substance, can be distinguished according to the ability to make use of various probable precursors instead of an end product. This is evidence that a mutant, incapable of growth let's say at the expense of P_2 but developing normally in media with P_3 and P_4, often forms a considerable amount of P_2, conclusively attesting to the advantage of the hypothesis concerning the fermentation blockade in the chain of the biochemical reactions."

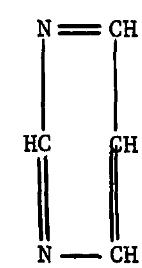
Works connected with the structure of the classification of the genus Piricularia verify the correctness of Beadle's system. Suryanarayanan (1958b) for example, showed that P. oryzae, as well as other species (P. setariae, P. zingiberi and Piricularia sp. on Eleusine coracana and Piricularia sp. on Triticum vulgare and others, are not able to synthesize vitamin B_1 (thiamine) themselves, but if pyrimidine is introduced into the medium, that is a portion of a molecule of thiamine (according to Beadle's system - this is the certain substance P_3), then the fungus will be able, by means of a whole series of biochemical reactions, to build a meager thiazole portion (substance P_2) of a molecule of thiamine and will grow normally (see schematic).



Molecule of thiamine • HCl



Molecule of thiazole



Molecule of pyrimidine

Only P. oryzae possesses such a capability to a full degree. It may be proposed that P. oryzae is the fungus with the best developed and most easily adapted fermentation apparatus. However even with the possession of such a wide collection of ferments, P. oryzae was not able to grow on Richard's medium with a thiazole component alone (see table 3). The capability of P. oryzae to grow in the presence of pyrimidine alone is exhibited only 6 days after the onset of growth. This means that the adaptive reorganization of the fungus doesn't take place immediately. Apparently a definite period of time is necessary for the formation of the ferment which catalyzes the process that finishes the building of the thiazole portion of the molecule.

The general theoretical foundation of Beadle's classification found practical application in the differentiation of fungi according to their

reaction on thiamine, which was proposed by Robbins - Hawker (1950).

All fungi, depending on their synthetic activity, were divided into four groups: 1) fungi, deficient in the entire molecule of thiamine and not capable of synthesizing this vitamin from its components (genus Phytophtora, for example; 2) fungi, capable of synthesizing thiamine from two components (for example, species of the genus Phycomyces); 3) fungi, capable of building the thiazole portion in the event a pyrimidine fraction is added to the medium (Phythium butleri and others); 4) fungi, capable of building pyrimidine in the event of nourishment with thiazole (Mucor ramanianus).

In conformity with the results obtained for species of the genus Piricularia, Suryanarayanan considers that P. oryzae may be placed in the third group and the remaining species of Piricularia in the second group.

P. oryzae can synthesize thiamine from pyrimidine alone if it is growing on glucose and maltose. In the event xylose and fructose are used, thiamine synthesis is suppressed and growth is absent in the fungi.

The basic facts supporting th accuracy of what has been stated are presented in table 3 (taken in abridged form from the works of Suryanarayanan, 1958b). For P. oryzae a different activity is also noted when various sugars are admitted into the medium. The classification was worked out for isolates of Piricularia taken from Oryza sativa, Setaria italica, Eleusine coracana, and Digitaria marginata, based on their relationship to sources of carbon. For example, it was shown that P. oryzae grows best of all on glucose, saccharose and maltose, and worse on cellulose and soluble starch (Ramakrishnan, 1948). Differentiation of the genus was also carried out according to the nitrogen scale - the fungus grew best of all on peptone and other organic sources of nitrogen and worst of all on nitrate.

III. Classification Within the Species Piricularia oryzae Cav. Based on Biochemical Characteristics

In as much as Piricularia oryzae Cav. is unusually aggressive and inflicts great damage to the agricultural economy of India, Japan, the USA, Italy, Hungary and a number of other countries, the study and classification of this species received particularly great attention. Leaver (1947) and Apparao (1955) showed that in the absence of thiamine and biotin in a synthetic medium there was no growth and sporulation in Piricularia. Pantothenic acid, riboflavin and nicotinic acid had no influence on growth and sporulation. It turned out that thiamine is an indispensable and obligatory component of a synthetic medium in the cultivation of this fungus. However, Otsuka and others (1958) reported that 38 out of 47 strains of P. oryzae which were studied were able to grow in a medium in which thiamine was replaced by nicotinic acid. The remaining 9 strains grew well in media with lowered thiamine content and produced a noticeable amount of nicotinic

acid. These observations are fully in agreement with Cohraine (1958) who considers that fungi which do not require anything from the vitamins are themselves capable of synthesizing it. Freshly synthesized vitamins usually appear in a cultured liquid (Otsuka, 1957).

Based on the specific relationship to thiamine, Otsuka divided the strains which he had into 13 groups.

A revision of the assumption concerning the indispensability of biotin for the growth and sporulation of P. oryzae also emanated from Otsuka (1958).

He showed that several strains are able to grow on asparagine just as well as on biotin; a deficiency of the later in the medium was successfully made up for by asparagine. Several strains didn't synthesize biotin at all and didn't require it. Another group of strains also didn't require biotin, but not because it wasn't needed for their growth but as a result of the fact that they themselves synthesized it.

The ability of the fungus P. oryzae to synthesize thiamine was studied against a background of various levels of hydrocarbon and amino acid nourishment. A whole series of hetero- and homo-trophic strains were checked for synthetic activity. For the quantitative determination of synthesized vitamins, they used microbiological tests - sensitive isolates of Lacto - bacillus arabinosis and Sacharomyces carlsbergensis, which were capable of reacting to insignificant amounts of thiamine in the medium (Otsuka, 1959).

Thanks to such a careful selection, simple strains were developed which were capable of growing excellently on media without biotin and thiamine. This made it possible for Japanese investigators to study the immunity of rice by using pathogenic organisms with an unusually high synthetic activity.

Otsuka (1957) and Suryanarayanan (1958a) showed that in the formation of an organism with a high synthetic activity, the nature of carbohydrate exchange changes sharply. New products of a type of nicotinic acid are formed; the usual methods of synthesis are displaced, for example the synthesis of malic, citric and malonic acids takes place apart from Kreb's cycle.

Differentiation of species was also carried out according to a number of other properties. Thus Neufeld and others (1956) attempted to classify the strains available to them according to the activity of the polyphenol-oxidase of the mycelium. This is especially interesting if it is taken into account what attention is payed to polyphenol compounds of the rice plant during the study of its reaction to the injection of Piricularia. It has been detected for example, that in low concentrations the toxin

secreted by the fungus stimulated, and in high concentrations suppressed the growth and respiration of rice. The introduction of chlorogenic acid completely relieved this suppression. Apparently the depressive action of the toxin is related to its influence on the system of polyphenols (Tamari, Kaji, 1955). Subsequently it was found out that immune varieties of rice are more resistant to the toxin, which is explained by the presence in them of chlorogenic acid, detected in vivo, and by the intensification of the synthesis of polyphenols which is carried out through a system of phosphorus containing carbohydrates (Ogasawara, 1957). Upon the formation of a host-parasite complex the respiratory systems are activated as well as the groups of carbohydrates involved in the new type of metabolism. By another of its aspects, carbohydrate exchange as we saw is intimately connected with the synthesis of vitamins. Therefore the endeavors seem completely justified which have been undertaken in the course of a number of years to classify P. oryzae accorring to reactions to carbohydrates. Tochinai (1940) demonstrated that some of the Japanese strains of this species utilized glycerine and mannitol in the capacity of sole sources of carbon, but absorbed mamitose, starch, glucose and pectin considerably better (citation according to Ramakrishnan, 1948). Inone (1939) cultivated 21 strains of P. oryzae on a cellulose medium and divided them, according to their activity in the breaking down of the substrate, into strong intermediate, and weak (citation according to Kulkarni and others, 1959). Kulkarni (1959) also obtained facts that the most virulent strain decomposed cellulose intensively. In 1959, 45 Japanese strains were divided according to the "carbohydrate scale". A large part of them utilized glucose, saccharose, maltose, fructose and lactose better and reacted poorly to the presence of inositol and salts of organic acids. However, the classification according to the "carbohydrate scale" proved insufficiently clear.

The pH was also one of the criteria for the classification of strains of the species P. oryzae. Nishikado (1927), while conducting differentiation of strains according to reaction to pH, established that this index can serve as a criterium of pathogenicity. The main group of P. oryzae strains available to Nishikado grew at a pH of from 5 to 10, however highly pathogenic strains grew better at a pH of 4.4.

Apparao (1956) also emphasized the importance of the pH for metabolism of fungus. He noted that the pH is a factor sharply influencing the utilization of nitrogen. It is possible that a low pH of the medium in a number of cases is responsible for the very weak growth of fungus. It followed then that fungi should be classified according to their capability to absorb ammonium and nitrate nitrogen. Differentiation of P. oryzae according to the relationship to sources of nitrogen is the second most absolute (after differentiation according to vitamins) classification within species.

A detailed study of the reaction of fungus to various sources of nitrogen preceeded the clear division of the Japanses collection of strains into groups. The most exhaustive information concerning this problem was given

by Nakamura and Shimomura (1953-1959). The basic theme of their investigation was the relationship of P. oryzae to amino acids. It was shown in an example of glutamic acid, that during the decomposition of amino acids there occurs the formation of a-keto-acids. Methods have been found for converting amino acids into keto acids and also the principles of transformation of one amino acid into another. These facts are in agreement with the results of the investigations of Apparao (1956) who noted that salts of organic acids stimulated the decomposition of ammonium ions by the fungus. He supposed that in the body of the fungus, organic acids and ammonium formed aspartic and glutamic acids. In this manner the transformation of organic acids and ammonium into amino acids and the reverse conversion of amino acids to keto-acids is closely related. The activity of the processes of deamination and transamination in the organisms of fungi of various strains was subsequently used in the capacity of a criteria in their classification. The connection has also been noted between the susceptibility of the plant and the content of amino acids in it. Thus the balance of glutamic acid in the plant represents the basic regulator of the intensity of affection by the Piricularia fungus (Apparao, 1956). During the period of active development of Piricularia in the rice, the activity of ferments of decarboxylase and transaminase is the greatest. This leads to the disturbance of the equilibrium in the outlay and accumulation of glutamic acid in the plant. Thus, the stability and susceptibility of rice is intimately related not only with the synthesis of polyphenols and carbohydrate exchange in the plant-host as shown above, but also with its nitrogen exchange. It is supposed that the amount of soluble nitrogenous substances formed in rice under the influence of fungus is strictly correlated with its aggressiveness (Suryanarayanan, 1958a).

A clarification of the various peculiarities of nitrogen exchange of the parasite and host was connected with exploratory experiments with the goal of a preliminary classification of strains. Leaver (1947) made the first attempt in this direction. He reported on the results of studying the influence of a whole group of nitrogenous substances on the growth of two isolates (table 4).

From this table it is plain that casein and glycine are the best sources of nitrogen for P. oryzae and hydroxylamine and ammonium chloride suppress its growth and sporulation. It must be noted that the two isolates studied reacted differently to one and the same source of nitrogen. This same author presents information concerning the sporulation of P. oryzae on a medium with amino acids. Six isolates were subjected to study (table 5).

Out of the five sources of nitrogen studied, glycine and tryptophan displayed themselves best of all as amino acid differentiators. According to Kulkarni (1959), who also differentiated P. oryzae by its relationship to amino acids, the poorest amino acid source of nitrogen for a virulent isolate was threonine, and for a weakly virulent isolate - tryptophan.

Thus glycine, tryptophan and threonine proved to be amino acid differentiators with the help of which it is possible to clearly separate out strongly and weakly sporulating forms. In 1958, Otsuka published an article in which it is reported that 45 strains were divided into 10 groups according to the capability of reducing nitrates and multiplying in a medium containing tryptophan and sodium nitrite.

Purine and pyrimidine bases and phasphatides are a special group of nitrogen containing compounds. Leaver (1947) used these substances for the differentiation of four basic isolates of fungus. One of the isolates was particularly lacking in choline, a second in guanine, and the fourth in xanthine. Leaver pointed out the great significance of these compounds for fungus. He presents facts, in conformance to which the above named compounds gave rise to the earlier sporulation of sterile strains.

Otsuka (1959) later established the direct tie between the content of nucleinic acids and nucleo proteides and the pathogenicity of Piricularia which supports the opinion of Leaver about the importance of the components of these compounds.

It must be pointed out that only as a result of many years work with P. oryzae which is described above, foreign investigators obtained clearly sorted material completely homologous to the conception of "physiological race".

Stakman, having established this term, considers that physiological races can be distinguished by intensity of growth, sizes and color (particularly in an artificial medium), by temperature and nourishment requirements, by fermentation activity, resistance to pH, poisons, fungicides and toxins, by the characteristics of forming substances toxic to plants, by pathogenicity and other properties (citation according to Kulkarni and others, 1959).

The selected material represented by physiological races with clear biochemical features can be obtained on the basis of their study and classification. Necessary for this are the identification and utilization of a large number of strains, an explanation of the most important stages of metabolism (by which these strains are distinguished) and the roles of individual groups of compounds in the growth and spore formation of fungus.

It must be pointed out one more time that during the conduct of these works consideration must always be given to the interrelationship of the plant-host and the parasite which made it possible for experimenters to classify, not abstract units, but forms of fungus with clearly determined pathogenic properties.

Conclusions

The history of the study of Piricularia fungus is interesting as an example of the deep and many sided approach to the problem of immunity. During the solving of what turned out to be an individual problem such as

combatting this parasite, the mechanism was exposed of the metabolism of healthy and diseased plants and also the physiological active substance emerging in the process of the interrelation of the fungus and host, and finally the basic requirements of the fungus in nutritive substances were studied in detail. Thanks to the vast factual material obtained by Indian and Japanese investigators, it was possible to successfully classify the existing forms of the fungus, having divided them into a number of clear groups with more or less permanent biochemical properties.

The present summary doesn't pretend to be a complete account. But it seemed important to us to sketch, even though in a general outline, a picture of the investigations of such an important and dangerous parasite as piricularia. Only a deep conception of all sides of its life activity makes it possible to effectively combat the spreading of rice disease and diseases of other agricultural crops caused by it.

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Literature

Abramov, I. N. (1928) Piricularia Fungus on Rice, Rice Blight., Izv. Yuzhno-Ussuriysk. otd. Gos. russk. geografich. obshch., 15.

Apparao, A. (1955), Growth requirements of Piricularia oryzae. Curr. Sci., 24, 4.

Apparao, A. (1956), The role of pH in nitrogen utilization by Piricularia oryzae. Experientia, 12, 6.

Atkins, J. (1956), An outbreak of Piricularia on rice in 1955. Plant Dis. Rep., 40, 5.

Beadle, G. (1945), Biochemical genetics. Chem. Rev., 37, 15 (Cited according to A. Klyuver, K. Van-Nil' (1960). Contribution of Microbes to Biology.).

Cohraine, V. (1958), Physiology of Fungi.

Corbetta, G. (1954), Concimazone potassicae azotata e malattie parasitarie del Riso. Riso, 2, 11.

Cralley, E., C. Adair. (1942), Rice blast in Arkansas. Plant Dis. Rep., 26.

Kulkarni, N., M. Patel and others, (1959), Physiological specialization of Piricularia oryzae in India. Poona Agric. College Mag., 49, 4.

Leaver, F., Leal a. C. Brewer (1947), Nutritional Studies on Piricularia oryzae, Journ. Bact. 54, 401.

Nakamura, J., Shimomura (1953-1959), Biochemical Studies of Piricularia oryzae. Bull. Agric. Soc. Japan, 16-22 (series of articles).

Neufeld, H. and others (1956), Polyphenol oxidase in Piricularia oryzae. Abs. in Fed. Proc., Part I, 15.

Nishikado, J. (1827) (Citation according to K. Ramakrishnan, 1948), Jap. Journ. Bot., 3, 3.

Ogasawara, N. (1957), On the biochemical studies of Piricularia oryzae. VIII. Journ. Agric. Chem. Soc. Japan, 31, 8.

Otsuka, H. and others (1957), On the biochemical studies of Piricularia oryzae. Journ. Agric. Chem. Soc. Japan, 31, 11.

Otsuka, H. and others (1958), On the biochemical studies of Piricularia oryzae. Journ. Agric. Chem. Soc. Japan, 32, 8.

Otsuka, H. and others (1959), On the biochemical studies of Piricularia oryzae. On the vitamin formation. Journ. Agric. Chem. Soc. Japan, 33, 12.

Otsuka, H. (1961), On the biochemical studies of Piricularia oryzae (cited according to the reference journal "Biological Chemistry", 1961, No. 5, 736)

Ramakrishnan, K. (1948), Studies on the morphology, physiology and parasitism of genus Piricularia in Madras. Ind. Acad. Sci., B, 27, 6.

Suryanarayanan, S. (1958a), Mechanism of resistance of paddy (Oryza sativa) to Piricularia oryzae Cav. Proc. Nat. Inst. Sci. India, 24, 6.

Suryanarayanan, S. (1958b), Thimine, its intermediates and growth of Piricularia spp. and Sclerotium oryzae. Phytopath. zeitschr., 33, 3.

Takachashi, J. (1956), Studies on the mechanism of resistance of rice plants. Bull. Jamagata Univ. Agric. Sci. 2, 2.

Tamari, K. a J. Kaji (1954), On the biochemical studies of the blast mould (Piricularia oryzae), the causative mould the blast disease of rice plant. I. Journ. Agric. Chem. Soc. Japan, 28, 3.

Tamari, K. a J. Kaji (1955), On the biochemical studies of the mould. Journ. Agric. Chem. Soc. Japan, 29, 3.

Tamari, K. a J. Kaji and others (1958), On the biochemical studies of the blast mould.., XI. Journ. Agric. Chem. Soc. Japan, 21, 2.

Tamari, K. and others (1960a), Biochemical studies on the blast disease of the rice plants, XIV. Journ. Agric. Chem. Soc. Japan, 34, 11.

Tamari, K. and others (1960b), Biochemical studies on the blast disease of the rice plants, XVI. Journ. Agric. Chem. Soc. Japan, 34, 11.

Terui, M. (1940), Internal formation of conidia of the rice blast fungus. Ann. Phytopath. Soc. Japan, 10, 2 - 3.

Uehara, K. (1958), On the production of phytoalexin by the host plant as a result of interaction between rice plant and the blast fungus. Ann. Phytopath. Soc. Japan, 23, 3.

Vamos, R. (1959), "Brusone" disease of rice in Hungary. Plant a. Soil., 11, 1.

Watanabe, F. (1957), Studies on the vaccine therapeutics of blast disease of rice plants, X. Ann. Phytopath. Soc. Japan, 23, 3.

Weintraub, R. and others (1957), Stimulation of germination of spores of Piricularia oryzae by sapogenins. Phytopath., 47, 1.

Weintraub, R. and others (1958), Chemical stimulation of germination of spores of Piricularia oryzae. Phytopath., 48, 1.

Yamada, H. (1959), The formation of mannitol by Piricularia oryzae. Biochem. et biolhys. acta, 33, 1.

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Table 1 (page 1319)

Ferments Formed by Piricularia Fungus

Ferment	Substrate	pH
Diastase	Starch	6.5
Inulase	Inulin	6.8
Lipase	Litmus-agar	7.3
Amidase	Asparagine	6.4
Trypsin	Egg albumen	6.4
Saccharase	Saccharose	6.5
Maltase	Maltose	5.9
Lactase	Lactose	6.3

Table 2 (page 1320)

Physiological Active Substances Produced by the Piricularia Fungi and the Plant-Host

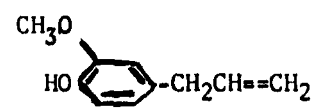
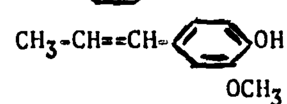
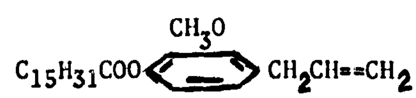
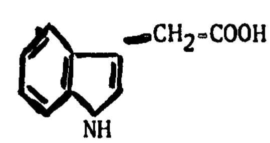
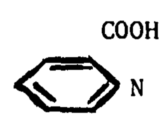
Name of Substance	Producer	Chemical Formula	Literary Citation
Phytoalexin	Rice & Fungus	Not established	Uehara, 1958
Orizarol		$C_{25}H_{44}O_3$	Tamari, 1958
			Tamari, 1960a, b
Isoeugenol	Rice		Tamari, 1960a, b
Eugenol-palmitate			Tamari, 1960a, b
3-indoleacetic acid			Watanabe, 1957
Piricularin, a & b	Fungus	$C_{17}H_{14}N_2O_3$	Tamari, 1954
a-Picolinic acid			Tamari, 1954

Table 3 (page 1322)

Growth Reaction of Species of the Genus Piricularia on Thiamine and Its Components

(weight 1 g per flask)

Composition of Nutrient Medium	P. oryzae	P. setariae	P. zingiberi	Piricularia sp. on Eleusine coracana
Control without components of thiamine	0.0	0.0	0.0	0.0
With thiazole	0.0	0.0	0.0	0.0
With pyrimidine	40.3	5.3	3.7	0.0
With pyrimidine and thiazole	41.0	33.3	46.5	29.9
With thiamine	38.4	33.7	44.1	27.9

Table 4 (page 1324)

Effect of Various Sources of Nitrogen on the Growth and Sporulation of Two Isolates of *Piricularia oryzae*

Source of Nitrogen	Growth	Number of Spores ($1000/cm^2$)	
		Isolate # 1	Isolate # 2
Casein	++++	235	200
Glycine	+++	409	369
Urea	+++	110	73
$P-NH_2$-benzoic acid	++	0	35
Uric Acid	++	78*	120
Caffeine citrate	++	78	32
Ammonium chloride	+	0	10
Without nitrogen	+	0	37
Hydroxylamine	-	0	0

* Translator's note: According to the original table (Leaver, 194[illegible]), this figure should be 6[illegible].9.

Table 5 (page 1324)

Effect of Various Amino Acids on the Sporulation of Piricularia oryzae

(number of spores in $1000/cm^2$) [1]

Source of Nitrogen	Subcultures (isolates)					
	1	2	3	4	5	6
Casein	310	247	55	101	203	111
Glycine	213	334	56	99	90	224
Tryptophan	83	134	179	56	104	201
Glutamic acid	101	103	128	125	119	120
Leucine	83	95	129	62	76	132

1 The table is abridged. In all the variants except the second one, a small amount of glycine was added to improve growth.